The University of Chicago

AFTER-RIPENING AND GERMINATION OF SEEDS OF TILIA, SAMBUCUS, AND RUBUS

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

BY
RIAL CATLIN ROSE

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AFTER-RIPENING AND GERMINATION OF SEEDS OF TILIA, SAMBUCUS, AND RUBUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 247

R. C. ROSE

Introduction

This paper gives the results of an attempt to determine the conditions favoring the after-ripening and germination of the seeds of *Tilia americana*, *Sambucus canadensis*, and *Rubus Idaeus*, and some of the chemical processes involved therein. Since layering of these seeds usually results in very low percentages of germination, it was thought possible to discover some other means of overcoming their dormancy.

Literature

The present state of our knowledge of the causes of delay in germination, and the means of overcoming it, is admirably summarized in a recent paper by CROCKER (5). He divides seeds which show delay in germination into 7 classes. In 3 of these the seed coats play the important rôle, while in the fourth dormancy is occasioned by the embryo. Where dormancy or poor germination is due to the seed coat, the use of concentrated sulphuric acid as a carbonizing agent has become a common practice. ROSE (23) mentions ROSTRUP (24) as the first to resort to this treatment, and lists TODARO (25), HILTNER (12), JARZYMOWSKI (14), BOLLEY (2), and LOVE and LEIGHTY (19) as investigators applying the same method. EWART (9) found this treatment effective with several

species of *Acacia*, as did CROCKER (unpublished work) with *Scirpus*. The length of time required by this treatment varies from a few minues to several hours, depending upon the resistance of the coats.

Boiling water or warm water, used as a forcing agent, has proved effective in a number of cases where hard-coatedness is the cause of the delay. BRUYNING (3), working with the seeds of *Ulex europaeus*, found that a treatment of 1–5 seconds with boiling water raised the percentage of germination from 13 for untreated seeds to 53·5–75·5 for treated seeds. HONING (13) obtained his best results with *Albizzia* seeds by soaking them in water at 60° C. for at least 3 hours, while with *Mimosa* 60–70° C. proved most effective, as did 70–75° C. for *Pithecolobium*. Soaking seeds of *Crotalaria* in warm water proved disadvantageous. BOLLEY (2) states that improvement in germination was obtained by this method if the exposure was not long enough to kill the embryo.

NOBBE (21) mentions ALEXANDER VON HUMBOLDT as the first investigator to use chemicals as forcing agents. From the time of HUMBOLDT (1793) up to 1873, the date of publication of NOBBE'S book, many investigators used as forcing agents a great variety of substances, both organic and inorganic. The range of substances used is more interesting than the results obtained. Moreover, quickly germinating seeds were used and in such cases the effect of forcing agents is not so striking as where dormancy is involved. Of the more recent workers in this field, LEHMANN (16) was the first to emphasize the importance of chemical substances in connection with germination. He showed that the seeds of *Ranunculus sceleratus* are forced into germination by Knop's solution, by soil, by soil wet with weak solutions of hydrochloric acid, potassium hydroxide, ferric chloride, and hydrogen peroxide. Two years later GASSNER (10) found Knop's solution effective on unthreshed seeds of *Chloris ciliata*, and more recently (11) has shown that for several other seeds various nitrogen compounds, especially nitrites and nitrates, are effective forcing agents. *Chloris ciliata* was found to have a membrane impermeable to potassium nitrate and magnesium nitrate, and from this GASSNER concludes that the effect is upon the seed coat alone. LEHMANN (17) and LEHMANN and OTTENWÄLDER (18), working with seeds representing

a number of different families, showed that acids in low concentrations, especially hydrochloric acid, are effective forcing agents. CROCKER and DAVIS (6) obtained similar results for seeds of *Amaranthus* (unpublished work) and *Alisma*. Bases are equally effective for *Sagittaria* and *Alisma*, but not for *Amaranthus*. According to OTTENWÄLDER (22) bases exert an inhibitory effect on seeds of *Epilobium hirsutum*.

In those cases where a state of dormancy exists in the embryo itself (*Crataegus* and *Malus*), temperatures slightly above freezing have been found effective in hastening after-ripening (7). In *Crataegus*, as ECKERSON (8) has shown, the hypocotyl becomes more acid as after-ripening progresses; hence dilute acids hasten after-ripening by acting upon the hypocotyl directly.

Material

The seeds used in these experiments were gathered in the summer or the fall of 1916 and 1917. Each year those of *Sambucus* were all collected on the same day from neighboring plants. *Tilia* seeds of the 1916 crop were collected during October from trees growing on the dunes at the southern end of Lake Michigan. The 1917 crop was gathered during September from trees in the parks of Washington, D.C. The seeds of *Rubus* were collected during late June 1916 from neighboring plants of several varieties, but no attempt was made to keep those of the different varieties separate. Among the seeds of all 3 species were found many without embryos or with defective embryos. In most cases this fact accounts for the varying number of seeds used in the cultures. Approximately 60 per cent of *Rubus*, 75 per cent of the 1916 *Sambucus*, and 80 per cent of the 1916 crop of *Tilia* were viable. Not more than 5 per cent of the 1917 crop of *Tilia* and *Sambucus* were defective.

Histology and microchemistry of seed coats

SAMBUCUS: *Endocarp.*—The seed in cross-section shows in the lignified endocarp 3 regions: (1) the outermost, consisting of 3 or 4 layers of cells of irregular size and shape, with thin walls and large lumina; (2) a middle one of 1 or 2 layers of fibers in cross-section; and (3) an inner one of 1 or 2 layers of fibers in longitudinal section. *Seed coat.*—This consists of several layers of collapsed cells with

lignified walls; the cells contain a considerable quantity of reducing sugar.

TILIA: *Pericarp.*—This is composed of two layers: (1) a surface region of loose fibers with cellulose walls, and (2) a thicker region of lignified fibers. *Seed coat.*—This consists of 3 regions: (1) cells with suberized or cutinized walls; (2) one layer of palisade cells with (a) outer end walls of cellulose, (b) a lignified light zone, (c) a pectinized region, and (d) a lignified region; and (3) 3 or 4 layers of cells with walls which stain with ruthenium red and give the ceric acid test.

RUBUS: *Endocarp.*—This consists of 2 layers: (1) an outer layer, variable in thickness, of lignified fibers longitudinally arranged in cross-section of the fruit; (2) an inner region of 4 or 5 layers of lignified fibers transversely arranged in cross-section of the fruit. *Testa.*—This consists of 4 regions: (1) 1 layer of cushion-shaped cells with lignified walls; (2) 4 layers of collapsed cells with cellulose walls; (3) 1 layer of collapsed cells with thick pectinized walls; and (4) 1 layer of cells with cellulose walls which appear as a thickened outer wall of the endosperm.

Microchemistry

In table I are given the results of the microchemical tests made upon the endosperm and embryo of each of the kinds of seeds used. Owing to the lack of a sufficient number of germinating seeds of *Sambucus* several of the tests have not been completed. The storage materials in all the seeds are very similar, starch, fats, and protein being found in every case. In addition to these *Sambucus* contains amylodextrin. *Tilia* contains much more fat and phytosterol than either *Sambucus* or *Rubus*. The phytosterol shows up as a bright red layer around the fat globules when sections of the seeds are placed in concentrated sulphuric acid. Oxidase is present in the dry seeds in very small quantities, and in the germinating seeds benzidine gives a positive test only after several hours. Peroxidase, while present in dry *Tilia* seeds, is much more abundant in the germinating seeds. Dry seeds of *Sambucus* and *Rubus* give no peroxidase reaction. Catalase is found in both dry and germinating seeds of all 3 species.

TABLE I

Substance	Tilia				Sambucus				Rubus			
	Ungerminated		Germinated		Ungerminated		Germinated		Ungerminated		Germinated	
	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo
Starch	+	++	++	+++	−*	++	−	++	−	−	+	++
Amylodextrin	−	−	−	−	++	−	+	−	−	−	−	−
Reducing sugar	−	−	+	++	−	−	?	?	−	−	+	+
Protein reaction:												
Xanthoprotein	+	+	+	++	++	++	?	?	+	+	+	+
Biuret	++	++	++	++	+	+	?	?	++	++	+	+
Fat	+++	++	++	+	+	+	+	+	+	+	+	+
Phytosterol	+++	++	++	+	+	+	?	?	++	++	+	+
Tannin	−	−	−	−	−	−	−	−	++	+	++	+
Oxidase	−	−	+	++	−	−	?	?	−	−	+	+
Peroxidase	+	+	++	++	−	−	+	++	−	−	+	++
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Reaction	Neutral or acid	Neutral or acid	Alkaline	Acid	Alkaline	Alkaline	Alkaline	Acid	Acid	Acid	Acid	Acid

Conclusive determinations in regard to the reaction of fresh dry seeds of *Tilia* have not been made, but preliminary tests, where neutral red was used as an indicator, indicate that the endosperm and cotyledons are acid and the hypocotyl alkaline. Seeds kept in dry warm storage for 9 months show an acid reaction throughout. Hydrogen ion determinations, the data for which are given later, showed an acid reaction for the stored seed as well as for the germinating ones. As germination begins, the reaction of the embryo of *Sambucus* changes from alkaline to acid, but the endosperm remains alkaline. Both dry and germinating *Rubus* seeds are acid. A qualitative analysis of the ash of *Tilia* seeds showed iron, calcium, magnesium, potassium, and aluminium present. No tests were made for sodium.

Experimental data

Freshly harvested *Tilia* seeds with a moisture content of 10 per cent or less, or seeds kept in dry warm storage for several months, fail to germinate when placed on a moist substratum and kept at room temperature. This is true not only of seeds with coats intact, but for those with the coats chipped or entirely removed. Fungi and bacteria soon attack seeds with the coats broken and decay takes place in a few days. The percentage of water held by air-dry seeds is shown in table II. The seeds used for these determinations were dried in a partial vacuum at 80° C. until the weight was constant.

TABLE II

WATER CONTENT OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water loss in gm.	Percentage of water loss
Coats off	1.2754*	0.0788	6.17
Coats on	1.8559	0.1782	9.60
Coats on	2.1904	0.1574	7.18
Coats on	1.5024	0.1130	7.52

*Average of 4 duplicates.

The variations in the percentage of water lost by the seeds with coats on is due to the presence of seed coats which contained no endosperm and embryo. That the failure of air-dry *Tilia* seeds,

coats either on or off, to germinate is not due to an inability to absorb water is indicated by table III. The data given in this table were obtained by soaking seeds in distilled water at room temperature until they had come to constant weight. Here again the

TABLE III

WATER-HOLDING CAPACITY OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water absorbed in gm.	Percentage of water absorbed
Coats off.....	1.2848*	1.2071	93.95
Coats on.....	2.1540	0.7841	36.40
Coats on.....	1.7842	0.4146	23.24
Coats on.....	2.1198	0.4804	22.66
Coats chipped.....	1.4963	1.5020	100.38
Coats chipped.....	1.5040	1.5717	104.50
Coats chipped.....	1.9590	1.9185	97.93

*Average of 4 duplicates.

variations in the percentage of water absorbed are in part due to the presence of seed coats which contain no endosperm and embryo. Even with the coats chipped it is not always possible to eliminate all empty coats or defective seeds. The fact that the coats interfere with water absorption to a considerable extent is clearly shown in the table. The fact that seeds with coats removed or chipped, however, and with a moisture content approximately equal to their air-dry weight will not germinate when placed on a moist substratum at room temperature, is sufficient proof that water absorption is not the only limiting factor to growth.

That seeds that have been stored in the air-dry condition when the seed coats are intact can be forced to germinate is shown by the following experiment. Approximately 7000 seeds (200 gm.) of the 1916 crop, with pericarps removed and coats chipped, were placed on moist cotton in large Petri dishes and kept at 4–6° C. from March 24, 1917, to June 10, 1917, a total of 78 days. At the end of that time and before being transferred to a higher temperature, several hundreds showed the hypocotyl protruding from the endosperm for 1.5–2.5 cm. Of these, 100 were planted in soil in the greenhouse and 71 per cent produced seedlings. A second lot of 100 seeds was planted in soil out of doors, and 64 per cent

produced seedlings. Two lots of 500 each were selected from the seeds in which the hypocotyl was still inclosed within the endosperm. These were planted in soil in the greenhouse and in the garden and gave 20 and 25 per cent germination respectively. All seeds not planted were again placed in cold storage. Twelve days later 400 with hypocotyls protruding from the endosperm were planted in soil in the greenhouse. Of these, 348, or 87 per cent, produced seedlings within a week. By July 24, 1666 of these 7000 cold storage seeds had germinated at a low temperature. Of the ungerminated seeds 100 placed on moist cotton at room temperature gave 31 per cent germination in one week. The roots of these were short and thick and showed a great tendency to coil. At the same time air-dry seeds which had been stored at room temperature, when placed in soil or on moist cotton, decayed. Seeds kept in cold storage showed for the first few days a great tendency to mold, so that it was necessary to sterilize them with a 3 per cent solution of hydrogen peroxide for 1 hour on two separate occasions. With longer storage an immunity toward fungi is established, and although the coats may be covered with a thick layer of mycelia the endosperm and embryo are not attacked. Sections of the seeds examined under the microscope failed to show any hyphae present within the living tissue. On November 6, 1917, 6 cultures of 50 seeds each of both the 1916 and 1917 crops were placed in moist storage at 0–2° C., where they were allowed to remain for 140 days. At the end of that time no germination had taken place, which is in direct contrast with the result obtained in 1916 with seeds stored at 4–6° C. The failure to obtain germination here is interpreted as being due to the use of too low a temperature. The assumption that the exposure to this temperature was too long will hardly explain the results obtained, since if the temperature were not too low germination should begin as soon as the after-ripening process is complete. The results given in table IV, showing the percentage of germination obtained when these seeds were transferred to a temperature of 10–12° C., indicate that the storage temperature and not the length of exposure to it is the limiting factor.

This conclusion is strengthened further by the following experiment. Unfortunately no count of the number of seeds germinated

was made, as the experiment was used primarily for a different purpose. Approximately 1000 seeds of each of the 2 crops, stored under the same conditions as those indicated in table IV, showed no

TABLE IV

SEEDS OF *Tilia* STORED AT 0–2°C. FOR 140 DAYS; THEN AT 10–12°C.

NUMBER OF CULTURE	PERCENTAGE OF GERMINATION AFTER			
	12 days at 10–12° C.		19 days at 10–12° C.	
	1916 seeds	1917 seeds	1916 seeds	1917 seeds
1	66	24	74	28
2	68	20	72	24
3	70	12	80	18
4	74	34	82	34
5	70	26	76	30
6	19	22	56	30

germination after 140 days at a low temperature. When brought to the higher temperature the 1916 seeds germinated vigorously and in large numbers for the first 12 days and until the hypocotyls were 2–3 cm. long. From this point on no development took place and the seedlings gradually died. Here a temperature of 10–12° C. seems to be too low for continued growth. The 1917 seeds germinated much less vigorously, in fewer numbers, and only a few developed hypocotyls 2 cm. long. Comparing the results obtained in 1916 with those obtained in 1917, it is seen that the seeds after-ripen and germinate at temperatures slightly above freezing. DAVIS and ROSE (7) working with *Crataegus* found that after-ripening takes place most rapidly at 3–6° C., and that temperatures considerably higher are more favorable for germination and growth. At 0–2° C. *Tilia* seeds after-ripen but do not germinate. At 4–6° C. after-ripening and germination both take place, the latter taking considerable time. After-ripened seeds germinate poorly at room temperature. Once germination has begun at the low temperature, growth is best at temperatures above 12° C. The germination of *Tilia* seeds depends, therefore, upon the proper regulation of the temperature, and can be accomplished by a period of after-ripening in moist storage at 0–2° C., followed by a sojourn of 2 or 3 weeks at 10–12° C. until germination is well under way,

and finally by a transfer to a still higher temperature in order to permit vigorous growth. These conclusions are drawn from the facts that (1) seeds after-ripened at 0–2° C. did not germinate until transferred to a temperature of 10–12° C.; (2) although germination began at the higher temperature, growth soon ceased; and (3) seeds which had been after-ripened and which had begun to germinate at 4–6° C. grew well when transferred to soil in the greenhouse. Table IV suggests that one-year old seeds are better than fresh, but additional data upon this point are desirable. A nurseryman with many years' experience in the growing of trees and shrubs states that if *Tilia* seeds are allowed to become dry between the time of maturing and the time of layering a low percentage of germination results. On the other hand, if a high moisture content is maintained during this period no difficulty in germination is encountered. Up to the present time the author has been unable to obtain seeds which at the time of gathering had a moisture content of more than 10 per cent, and it seems probable that the water content of *Tilia* seeds is generally low at harvest time. While these seeds do not after-ripen to any considerable degree in air-dry storage, those that have been in the air-dry condition for a year after-ripen perfectly when put in a moist germinator at a low temperature. There seems to be no injury, therefore, even from protracted air-dry storage. No discussion is necessary to show that field conditions are not those most favorable for the obtaining of high percentages of germination. Neither does the nurseryman, when layering seeds, control the temperatures to the extent necessary to secure maximum results.

HYDROGEN ION CONCENTRATION.—The determinations of the hydrogen ion concentrations were made with the hydrogen electrode. Twenty seeds were pulverized in a mortar, and, except in instances to be noted later, 25 cc. of water added. The temperature varied from 27 to 33° C., but in every case the necessary correction was made. The determinations were made upon the seeds in the unafter-ripened condition, after-ripened but not germinated, with hypocotyl 2 mm. to 5 mm. long, and with hypocotyl 0.5 cm. to 2 cm. long.

ECKERSON (8) has already shown that the acidity of the hypocotyl of *Crataegus* increases as after-ripening progresses. Her

determinations were made by the titration method with phenophthalein as an indicator. The P_H of seeds with the hypocotyls 0.5 cm. to 2 cm. long is approximately 4 times as great as that of the unafter-ripened seeds. While this is not as great an increase as that found by ECKERSON, it may be due to the fact that her determinations were made upon the dormant organ alone, while here the whole seed was used, or to differences between the two kinds of seeds. Determinations made by the titration method would also probably give values much higher than those obtained by the hydrogen elctrode.

Table V shows that the weight of the seeds increases as after-ripening progresses. This is not due to an increase in dry weight,

TABLE V

CONCENTRATION OF HYDROGEN ION OF *Tilia* SEEDS IN DIFFERENT STAGES OF AFTER-RIPENING

	Condition of seeds	Weight in gm.	P_H
1	Air-dry	0.384	2.24×10^{-7}
2	Air-dry	0.443	2.00×10^{-7}
3	Air-dry	0.379	2.58×10^{-7}
4	Air-dry	0.379	2.40×10^{-7}
5	After-ripened	0.714	3.24×10^{-7}
6	After-ripened	0.752	3.02×10^{-7}
7	With hypocotyls 5 mm.	0.943	6.76×10^{-7}
8	With hypocotyls 5 mm.	0.932	5.75×10^{-7}
9	With hypocotyls 5 mm.	0.946	7.59×10^{-7}
10	With hypocotyls 0.5–2 cm.	1.553	1.18×10^{-6}
11	With hypocotyls 0.5–2 cm.	1.638	1.10×10^{-6}
12*	With hypocotyls 0.5–2 cm.	1.578	9.33×10^{-7}
13*	With hypocotyls 0.5–2 cm.	1.709	9.33×10^{-7}
14†	With hypocotyls 0.5–2 cm.	1.450	1.05×10^{-6}
15†	With hypocotyls 0.5–2 cm.	1.571	1.05×10^{-6}
16	After-ripened at room temperature (10 days)	0.689	2.51×10^{-7}
17	After-ripened at room temperature (10 days)	0.642	2.51×10^{-7}

* 50 cc. of water used.

† 100 cc. of water used; 25 cc. of water used for all others.

since no photosynthesis has taken place, but to the large amount of water absorbed. ECKERSON (8) likewise observed an increased water-holding capacity for the hypocotyl of *Crataegus* as after-ripening progressed. Of greater significance in this connection is the fact that, at least for the most advanced stage of after-ripening, variations in the amount of water used with the sample had little

effect upon the hydrogen ion concentration. With samples 10 and 11, 25 cc. of water were used, with samples 12 and 13, 50 cc., and with samples 14 and 15, 100 cc. Although the variation of P_H is considerable, it is by no means as great as that of the amount of water used, nor is it in the same direction. That the degree of dilution has no effect upon the P_H suggests the presence of buffer salts, formed by the action of fatty acids produced during germination and the constituents of the ash already mentioned.

After-ripened seeds similar to those used in samples 5 and 6, which had failed to germinate when kept at room temperature for 10 days, gave a P_H corresponding very closely to that shown by unafter-ripened seeds. This suggests that after-ripening is a reversible process, a fact to which CROCKER (5) has called attention, and that a decrease in acidity may lead to secondary dormancy.

TITRATABLE ACID.—Determinations of the titratable acid were made upon dry, after-ripened, and germinating seeds. For each determination the seeds were ground in a mortar with 10 cc. of water and titrated with N/10 sodium hydroxide with phenophthalein as an indicator. Titrations were made with freshly prepared samples and with others which had been allowed to stand for 48 hours. To the latter were added 10 drops of toluol and 0.5 cc. of N/10 hydrochloric acid. Table VI shows the number of cubic centimeters of N/10 sodium hydroxide necessary to neutralize the free acid in each sample. The figures are the average of duplicate determinations. Corrections have been made for the acid added.

TABLE VI

Condition of seeds	Fresh samples	After 48 hours	Percentage of increase
Dry	0.41	0.87	112.2
After-ripened	0.45	1.87	315.5
Germinating	1.18	2.80	137.2

While the amount of acid present is greatest in germinating seeds, it is seen that after autodigesting 48 hours the greatest percentage of increase over the freshly prepared samples is in seeds well after-ripened. Here is shown the fact that the after-ripened

seeds have a great power of increasing their alkali absorption, which may be due to lipase activity.

CATALASE.—Determinations of catalase activity of dry, after-ripened, and germinating seeds were made by means of APPLEMAN'S apparatus (1). The samples, ground in a mortar, were all reduced to the same degree of fineness by rubbing them through bolting cloth. The catalase determinations were made at 25° C. To 5 cc. of water containing 0.02 gm. of pulverized seed material was added 5 cc. of Oakland dioxygen and the amount of oxygen released was measured after 1, 2, 3, and 5 minutes of activity. APPLEMAN has pointed out that small amounts of acid greatly reduce or entirely destroy catalase activity. In order to remove this possible source of error the Oakland dioxygen used was neutralized by the addition of N/10 NaOH, or an excess of $CaCO_3$ was added to the meal. The data given in table VII are the averages of duplicate determinations. They show that dry, after-ripened, and germinating seeds, in the order named, exhibit increasing catalase activity. ECKERSON (8), employing microchemical methods, arrived at similar conclusions for seeds of *Crataegus*.

TABLE VII

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
1. Dry seeds	Neutralized*	2.5	4.2	5.25	7.0
2. After-ripened (dried 2 days)	Neutralized*	7.1	11.8	15.4	21.5
3. After-ripened (dried 2 days)	With $CaCO_3$	6.8	11.9	14.8	21.05
4. After-ripened (not dried)	With $CaCO_3$	6.75	11.3	15.05	20.75
5. Germinating	With $CaCO_3$	19.4	27.86	31.5	37.03

*0.80 cc. N/10 NaOH to neutralize 25 cc. dioxygen.

Drying after-ripened seeds for 2 days at room temperature has no effect on the amount of oxygen liberated, as is shown by comparison of samples 3 and 4.

Further evidence for the effect of the acid of the dioxygen upon catalase activity is shown in table VIII. Determinations made with after-ripened seeds not dried and with germinating seeds gave similar results. A comparison of the last 2 determinations show

that the neutralization of dioxygen or the addition of $CaCO_3$ is sufficient to eliminate any error due to the acidity of the reagent or the meal.

TABLE VIII

EFFECT OF REACTION OF SOLUTION UPON AMOUNT OF OXYGEN LIBERATED FROM DIOXYGEN BY *Tilia* SEEDS

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
After-ripened (dried 2 days)....	Not neutralized	2.1	3.1	3.6	4.3
After-ripened (dried 2 days)....	Neutralized	7.1	11.8	15.4	21.5
After-ripened (dried 2 days)....	With $CaCO_3$	6.8	11.9	14.8	21.05

OXIDASE ACTIVITY.—The determinations of oxidase activity were made on dry, after-ripened, and germinating seeds in BUNZELL'S (4) simplified apparatus with pyrogallol as the reagent. The material used, except in the case of the dried seeds, had been after-ripened at 0–2° C. for 140 days and then kept at 10–12° C. until a large percentage of the seeds had begun to germinate. After being dried in a vacuum over lime for 3 days at room temperature the seeds were ground in a mortar and the determinations made on 0.02 gm. of meal. Table IX shows the readings in centimeters of mercury after 3 hours and after 20.5 hours.

TABLE IX

OXIDASE ACTIVITY OF DRY, AFTER-RIPENED, AND GERMINATING SEEDS OF *Tilia*

Time	Dry seeds	After-ripened	Hypocotyls 1–5 mm. long	Hypocotyls 0.5–2 cm. long
After 3 hours.....	0.52	1.03	2.01	1.39
After 3 hours.....	0.53	1.10	1.92	1.52
After 20.5 hours.....	0.67	1.52	2.57	2.42
After 20.5 hours.....	0.68	1.67	2.52	2.07

During the experiment the temperature averaged 31.3° C. with a variation of ±0.1 of a degree. Variations in the volume of air in the tubes due to this slight variation in temperature have been corrected by means of check tubes containing water only. The

results show that the oxidase activity rises with after-ripening and germination. Once germination has begun, no increase is to be noted.

Discussion.—The results obtained show that the dormancy exhibited by the seeds of *Tilia* is not due to any property of the seed coat, although that structure may serve to lengthen the dormant period, but is to be ascribed to conditions obtaining within the endosperm or the embryo or both. In this respect *Tilia* resembles *Crataegus*, and the conditions necessary for after-ripening and germinating of the former are very similar to those required by the latter. Even with these conditions well known and various differences between dormant and after-ripened seeds clearly shown, it is still impossible to define the term after-ripening in anything more than general terms. The similarity of *Tilia* and *Crataegus*, with respect to the conditions necessary for after-ripening, does not permit one to conclude that the process in the two is the same. In any case after-ripening is not to be attributed to a change in any one condition, but to a series of changes which may vary for each individual case. Dormancy is to be looked upon, perhaps, as a condition of equilibrium in a series of chemical reactions; after-ripening as a displacement of this condition. Why low temperatures are effective in causing these changes and why the range of effective temperatures is so narrow are questions still to be answered.

Sambucus

Kinzel (15) states that for *Sambucus nigra* freezing for 2 winters is sufficient to bring only 39 per cent of the seeds to germination. Even longer freezing is necessary for the seeds of *S. racemosus*. Results obtained by the writer in experiments to be described are very similar to those given by Kinzel, and show that in neither case have the conditions necessary for germination been even approximately determined.

Nurserymen claim that layering results in almost perfect germination if the seeds are not allowed to become dry between the time of maturing and the time of layering. Air-dry seeds are considered worthless. These statements are in a large measure

confirmed by the following experiments, although sufficient data are not yet available to warrant a final statement.

Seeds removed from berries and allowed to dry at room temperature for 2 days failed to germinate within 2 weeks when placed on moist cotton, although they never contained less than 22 per cent of moisture. Fresh seeds on moist cotton kept at 4–6°, 0–2°, or 8° C. have never given more than 20 per cent germination when placed at room temperature or above. Air-dry seeds have given no better results. Although these seeds were kept at the low temperature for not less than 2 months, a longer period may be necessary. The experiments show that failure to germinate is not entirely due to injury resulting from drying, although that may be one of the determining factors. Neither is it to be attributed to inability of air-dry seeds to absorb water, since the quantity taken up in 48 hours by seeds with coats intact is equal to 38.55 per cent of their air-dry weight, while seeds with coats punctured absorb 39.16 per cent. Air-dry seeds contain approximately 6 per cent of water.

The effect of layering is shown by the following experiments, in which the number of seeds used for the 1916 crop was 1000 and for the 1917 crop 5000. Two lots of air-dry seeds of the 1916 crop were mixed with soil. One lot was kept at 15–20° C., the other out of doors over winter. In spring the percentages of germination were 8 and 44 respectively. Fresh seed of the 1917 crop, which had not been permitted to become dry when treated in the same way, gave 51 per cent and 77 per cent respectively. Air-dry seeds of the 1916 crop one year old failed to show any germination. Loss of water seems to be accompanied by a reduction in vitality.

Air-dry seeds gathered on October 14, 1916, were treated within 30 days with weak solutions of a large number of acids, bases, and salts. The acids used were malic, citric, tartaric, acetic, and butyric; the bases, potassium hydroxide, ammonium hydroxide, and sodium hydroxide; and the salts, sodium sulphate, nickel sulphate, ammonium sulphate, zinc sulphate, potassium sulphate, potassium nitrate, sodium nitrate, cobalt nitrate, ammonium nitrate, calcium chloride, sodium chloride, barium chloride, and potassium thiocyanate. The dilutions of the acids were N/200

and N/400; of the bases, N/1000, N/2500, N/5000, and N/10,000; and of the salts, N/20 and N/200. The number of perfect seeds in the cultures varied from 43 to 96. In only 4 cases was the number below 60, and the average was 75. This variation is due to the presence of empty seed coats which could not be distinguished from the perfect seeds until they had taken up a considerable quantity of water. It was later found possible to candle the seeds and thus eliminate the majority of the empty coats. The candling was done by means of an incandescent light supported below a glass plate upon which the seeds were placed. Between the light and the plate was placed a vessel of water to prevent undue heating. The seeds were placed in 20 cc. test tubes containing the solutions and allowed to soak for 24 hours. At the end of that time the solutions were drawn off and the seeds distributed over the moist walls of the test tubes, which were then plugged with cotton and kept at a temperature varying from 4 to 23° C. As soon as the seeds began to show signs of germination, they were removed from the tubes and placed in Petri dishes on moist cotton and kept at room temperature. Germination was slow, in the majority of cases extending over a period of 3 months. In the case of acetic acid, N/400, 58 per cent of the seeds germinated at the end of 176 days. The acids other than acetic showed little effect. The length of time over which bases can have any effect must be short, since in dilute solutions they are soon neutralized by the carbon dioxide of the air and that produced by the seeds. The cultures which showed germinations equal to or better than the checks are listed in table X.

In order to test the effect of constant low temperature upon seeds soaked in solution of various chemicals, a second set of cultures was prepared in the manner already described and kept at 4–6° C. for 63 days. At the end of that time the tubes were placed at room temperature. To the list of substances used in the preceding experiment were added potassium citrate, potassium tartrate, potassium acetate, potassium chlorate, ammonium nitrate, potassium iodide, lithium chloride, ammonium chloride, magnesium chloride, sodium nitrite, and dipotassium phosphate, and also hydrochloric acid and sulphuric acid. The concentrations of the mineral acids were N/1000, N/2500, N/5000, N/10,000, and of the

salts N/20, N/200, and N/1000. Germination began 4 days after the cultures were placed at room temperature and continued for 18 days. At the end of that time in practically all of the cultures, in addition to the seeds which had germinated, others were found

TABLE X

Sambucus SEEDS IN DILUTIONS OF ACIDS, BASES, AND SALTS; TEMPERATURE 4–23° C.

Substance	Normality of solution	Number of seeds	Percentage of germination
Distilled water	.....	78	12
Distilled water	.....	85	10
Distilled water	.....	68	10
Distilled water	.....	83	13
Acetic acid	N/200	79	18
Acetic acid	N/400	72	28
Malic acid	N/400	75	15
NH_4OH	N/2500	77	18
NaOH	N/1000	88	19
NaOH	N/2500	70	17
$(NH_4)_2SO_4$	N/20	75	28
$(NH_4)_2SO_4$	N/200	67	15
$ZnSO_4$	N/20	50	22
KNO_3	N/200	80	30
$NaNO_3$	N/20	46	19
$NaNO_3$	N/200	72	30
$CoNO_3$	N/200	59	71
KCNS	N/200	66	31

with the seed coat ruptured, but showing no sign of growth. All cultures in which a forcing effect of the solution is indicated by the germination of 20 per cent or more of the seeds are listed in table XI.

Out of 13 other substances not given in the table, 5 showed results equal to or better than the average of the checks in at least one dilution. The nitrates and sulphates are again found among the more effective substances. So far as the nitrogen compounds are concerned, these results agree with those of GASSNER (10) for seeds of *Chloris ciliata*.

Potassium nitrate, mercuric chloride, and potassium iodide used in connection with alternating temperatures had even less forcing effect than the substances given in table XI. The concentrations used were for potassium nitrate N/20, N/100, N/200, N/500,

N/1000, N/2000; for mercuric chloride N/400, N/1000, N/2000, N/4000, N/10,000; and for potassium iodide N/20, N/100, N/500, N/1000, N/2000. Three sets of cultures were set up in duplicate.

TABLE XI

Sambucus SEEDS IN ACIDS, BASES, AND SALTS

Substance	Normality of solution	Number of seeds	Percentage of germination	Percentage of seeds with ruptured coats	Total percentage of seeds affected
HCl	N/5000	65	17	6	23
H_2SO_4	N/2500	66	18	10	28
H_2SO_4	N/10,000	63	15	6	21
NH_4OH	N/1000	89	23	16	39
NH_4OH	N/5000	96	13	8	21
$C_6H_8O_9$	N/400	101	21	4	25
KNO_3	N/20	78	28	25	53
KNO_3	N/200	77	13	18	31
KNO_3	N/1000	86	24	8	32
$CoNO_3$	N/200	92	4	44	48
$CoNO_3$	N/1000	87	24	23	47
NH_4NO_3	N/200	108	14	39	53
NH_4NO_3	N/1000	56	14	12	26
$NaNO_3$	N/20	96	20	18	38
$NaNO_3$	N/200	83	33	20	53
$NaNO_2$	N/200	72	25	26	51
$NaNO_2$	N/1000	65	9	18	27
Na_2SO_4	N/200	94	10	10	20
$NiSO_4$	N/20	84	10	10	20
$NiSO_4$	N/200	86	7	34	41
$NiSO_4$	N/1000	76	15	10	25
$(NH_4)_2SO_4$	N/200	85	5	37	42
$ZnSO_4$	N/20	86	41	1	42
KCL	N/20	80	0	22	22
LiCl	N/1000	80	0	22	22
NaCl	N/200	89	1	37	38
NH_4Cl	N/20	97	2	24	26
NH_4Cl	N/200	84	13	15	28
Potassium citrate	N/20	84	0	21	21
Potassium citrate	N/1000	95	7	37	44
$KClO_3$	N/1000	81	9	18	27
KI	N/20	89	10	33	43
KCNS	N/200	90	9	12	21
K_2HPO_4	N/20	83	1	24	25
K_2HPO_4	N/1000	55	0	34	34
Distilled water	.....	70	0	7	7
Distilled water	.....	85	8	7	17
Distilled water	.....	109	1	14	15

One set was kept at 20° C. and a second at 30° C. The third set was kept at 20° C. for 18 hours and at 30° C. for 6 out of every 24 hours. The air in the tubes was changed every second day. The

duration of the experiment was 38 days. At the end of that time the only germinations obtained were those in the potassium nitrate, and in no case did these exceed 4 per cent. The seeds in the stronger mercuric chloride solutions were killed.

The rôle played by the coat in the behavior of the seeds has not been determined. Of naked embryos placed on moist cotton 32 per cent developed chlorophyll within a week, formed the hypocotyl arch, and attained a length of 5–10 mm. Naked embryos previously soaked in dilutions of hydrochloric acid and butyric acid and then placed on moist cotton gave no better results.

Seeds treated with concentrated sulphuric acid for 4–60 minutes and then kept under various conditions in regard to light, temperature, and oxygen pressure have never given over 20 per cent germination. A slight forcing effect by low concentrations of sulphuric acid was observed on seeds previously treated with concentrated sulphuric acid for 2–14 minutes and kept in the light at room temperature. Seeds immersed for 5 minutes in water at 40° C. in 55 days gave 25 per cent germination. Reheated at the same temperature for 3 minutes, 33 per cent germinated after 40 days. Longer heating at 40° C. or up to 70° C. gave lower percentages of germination. Untreated seeds gave no germination in the same length of time.

These results emphasize the following facts concerning the conditions necessary for the germination of *Sambucus* seeds: (1) air-dry seeds with a moisture content of 6 per cent or fresh seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum at room temperature; (2) this is not due entirely to injury resulting from drying, although that may be one of the determining factors; (3) air-dry seeds are able to absorb water to the extent of approximately 40 per cent of their air-dry weight, indicating that failure to germinate is not due to lack of water; (4) the effect of chemicals upon air-dry seeds is not marked, a slight forcing effect of several acids, bases, and salts has been observed, among which substances are found nitrates and sulphates; (5) the rôle played by the coat in the behavior of the seed has not been fully determined; (6) a slight forcing effect by low concentrations of sulphuric acid and by water at 40° C. has been observed;

(7) seeds remaining in contact with moist soil out of doors over winter gave 77 per cent of germination the next spring; whether this result is due to the low temperature, to certain constituents of the soil, or to a combination of these or other factors one cannot say.

The results obtained by KINZEL (15), together with those just summarized, show that as yet the conditions necessary for the germination of *Sambucus* seeds are not fully determined. To permit the water content of the seeds to fall below an undetermined critical point may lessen their viability. However, that some other condition or combination of conditions is responsible for the low percentages of germination must not be overlooked. KINZEL's suggestion that prolonged freezing is necessary should be given due consideration.

RUBUS

Seed fruits of *Rubus Idaeus*, like the seeds of the 2 species already discussed, fail to germinate when placed on a moist substratum. It was determined that this is not due to an immature condition of the embryo. If the pericarp is left intact all treatments with low concentrations of acids, bases, and salts, immersion in warm water, cold storage, exposure to increased oxygen pressure, or to ether vapor, freezing and thawing, and injection with water under pressure are ineffective.

When buried in the soil at 15–20° C. or out of doors over winter, a low percentage of germination takes place if the seeds are kept moist. Two lots of 720 viable seeds buried for 140 days under these conditions gave respectively 40 per cent and 20 per cent germination. Of 2 similar lots of seeds buried in tightly stoppered bottles, one at constant, the other at varying temperatures, none germinated when planted in the soil in the greenhouse. That these results are not due to injury resulting from drying or to inability to absorb water is indicated in table XII. The removal of the endocarp was accomplished by soaking the seeds in concentrated sulphuric acid for approximately 2 hours. Following this treatment the seeds were washed quickly in a large amount of running water to prevent heating, then immersed in a 5 per cent solution of sodium bicarbonate to neutralize the remaining acid,

and finally rinsed in running water for 15 or 20 minutes. The carbonized endocarp was removed by rubbing the treated seed on filter paper. The selection of perfect seeds was now an easy matter.

Table XII shows that the water-absorbing power for the seeds with the endocarp removed is 36–37 per cent of their air-dry weight, while that for the seeds with the endocarp intact reaches

TABLE XII

WATER CONTENT AND WATER HOLDING CAPACITY OF *Rubus* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Weight of seeds dried in vacuum at 75° C.	Percentage of water in air-dried seeds	Weight of soaked seeds in gm.	Water absorbed by air-dry seeds in gm.	Percentage of water absorbed by air-dry seeds
Endocarp removed .	0.6686	0.5842	12.62*	0.9120	0.2434	36.40*
Endocarp removed .	0.6864	0.6009	12.45	0.9414	0.2550	37.15
Endocarp intact....	1.0464	0.9328	10.85	1.5053	0.4589	43.85
Endocarp intact....	2.0960	1.8680	10.87	3.0080	0.9120	43.51

* On basis of air-dry weight.

almost 44 per cent of their air-dry weight. From this it follows that the water absorbing power of the endocarp is greater than that of the seed with the endocarp removed. There is no evidence to show that the endocarp possesses any structure which would prevent the water absorbed by it from being passed on to the seed. Although seeds with the endocarp intact will not germinate, when that structure is removed by means of the sulphuric acid treatment germination takes place within a few days, as is shown in table XIII.

The greater amount of the germination takes place between the fourth and tenth days. In seeds germinating after the tenth or twelfth day, growth is usually slow and the seedlings are weak. Failure to secure 100 per cent germination is due to the fact that during the removal of the carbonized endocarp in almost every case the seed coat is ruptured and the endosperm exposed to the attacks of bacteria and fungi. With the carbonized endocarp intact, uncertainty as to the extent to which the acid had penetrated and the inability to determine the number of fruits containing viable embryos lead to greater error than that occasioned by the attacks of the bacteria and fungi.

MÜLLER (20) has recently pointed out that in various seeds that germinate readily the outward pressure of the contents at the time of rupture was but slightly greater than the breaking strength of the water-saturated coat, and CROCKER and DAVIS (6) have found that seeds of *Alisma* are held in a dormant condition because the force of the expanding contents is not sufficient to rupture the coats.

TABLE XIII

SEEDS OF *Rubus Idaeus* WITH ENDOCARP REMOVED; 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

TREATED WITH ACID	PERCENTAGE OF GERMINATION AFTER				
	4 days	6 days	8 days	10 days	20 days
May 4	.....	.....	70	84	96
May 13	2	45	52	63	70
May 13	32	48	53	61	61
May 13	24	46	.....	55	55
May 24	.....	50	73	83	88
May 24	.....	20	63	77	84
May 24	.....	22	61	.....	80
May 24	.....	40	78	86	88
May 24*	.....	46	78	86	93
May 24*	.....	57	77	82	89
June 9	.....	50	.....	86	95

*In darkness.

Failure to absorb water is not the limiting factor, since both reach saturation after about 5 hours' soaking. Two facts indicate that *Rubus* seeds belong in the same class with *Alisma*. In the first place they germinate readily once the endocarp is removed, and in the second place even with the endocarp intact they absorb water readily. Occasionally ungerminated seeds with the endocarp removed have been found which when examined closely show no break in the coat. This suggests that the inner pectinized layer of the coat may play a part in the delay, either by limiting water or oxygen absorption, or both. As already indicated, the removal of the carbonized endocarp resulted in the rupture of the coat in practically 100 per cent of the seeds. This renders extremely difficult the determination of the part played by that structure.

Table XIV shows that the substrata most favorable for germination of naked seed are cotton, filter paper, and quartz sand. An

inhibitory effect is shown by garden soil, clay, and greenhouse soil, the effect of the last named being greatest. These soils acidified gave no better results. Calcium carbonate used on filter paper or in sand to neutralize any acid present in the medium or remaining on the seeds after the sulphuric acid treatment had no inhibitory effect. Glass wool moistened with a boiling water extract or a cold water extract of greenhouse soil cut down the percentage of germination to less than 50. Moreover, the seedlings were weak, with enlarged and discolored roots. In many cases germination started, but the roots were killed as soon as they came in contact with the substratum. Bone meal had been added to the greenhouse soil and this probably accounts for the injurious effect of the soil and the extracts. As is shown in table XIV, soaking in water for 24 hours

TABLE XIV

EFFECT OF SUBSTRATUM UPON GERMINATION OF NAKED SEEDS OF *Rubus Idaeus*; 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	25 days
Filter paper	50	77	86	90	95
Filter paper with $CaCO_3$	31	84	87	90	92
Quartz sand	48	80	83	85	92
Quartz sand	42	83	88	89	93
Quartz sand with 5 per cent $CaCO_3$	43	78	80	.....	88
Greenhouse soil	.....	.....	10	13	25
Greenhouse soil	.....	.....	.....	1	1
Greenhouse soil, acid	.....	.....	.....	1	1
Hot water extract greenhouse soil	4	22	41	.....	43
Cold water extract greenhouse soil	5	25	48	.....	48
Garden soil	5	25	.....	29	29
Garden soil, acid	1	9	.....	10	10
Clay	.....	3	9	11	19

previous to planting in garden soil raises the percentage of germination to 55. On the other hand, soaked seeds planted on moist cotton gave 71 per cent as against 72 per cent for unsoaked seeds. Germination was at practically the same rate in the two cases. Seeds planted on 5 per cent agar gave almost as high percentages as those on 1 per cent. The results given in tables XIV and XV show that the water supply is not the limiting factor.

Seeds in the soil are more exposed to attacks by fungi than those on agar or cotton. Previous soaking shortens the time the seeds must lie in the soil before germination begins, and hence lessens the chance for infection. Unsoaked seeds placed on moist cotton

TABLE XV

EFFECT OF WATER SUPPLY UPON GERMINATION OF SEEDS OF *Rubus Idaeus;* 100 SEEDS PER CULTURE; TEMPERATURE 20–25° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	16 days
1 per cent agar*	37	49	70	70	70
1 per cent agar*	22	50	.....	62	62
2 per cent agar*	30	37	50	50	50
2 per cent agar*	40	64	.....	73	73
5 per cent agar*	35	42	60	.....	60
5 per cent agar*	32	52	.....	61	61
Soaked 24 hours, then in garden soil	12	36	47	50	55
Soaked 24 hours, then on moist cotton	43	64	70	71	71
Not soaked, on moist cotton	32	66	72	.....	72

* Average of 2 duplicate determinations.

absorb water easily, hence swell more rapidly than in the soil, and moreover are less liable to infection. Under these conditions soaking offers no advantage.

Summary

GENERAL.—Air-dry seeds of *Tilia americana, Sambucus canadensis,* and *Rubus Idaeus* do not germinate when placed on a moist substratum at room temperature. In no case does water absorption seem to be the limiting factor. Air-dry seeds planted in the soil over winter give low percentages of germination.

TILIA.—Seed coats are not the cause of dormancy, although they may serve to lengthen the dormant period. A state of dormancy exists in the endosperm or embryo, or both.

Seeds with coats removed after-ripen at temperatures slightly above freezing. At 0–2° C. seeds after-ripen, but do not germinate. At 4–6° C. both after-ripening and germination take place. Seeds after-ripened at 0–2° C. germinate readily at 10–12° C., but very poorly at room temperature. Once germination has begun growth proceeds best at temperatures above 12° C.

As after-ripening progresses the hydrogen ion concentration increases, as do also the water holding capacity and the oxidase and catalase activities.

The greatest amount of free acid is present in the germinating seeds. Autodigestion of pulverized seeds shows the greatest acid increase in the after-ripened ungerminated seeds. This is probably due to their high lipase activity.

SAMBUCUS.—As high as 77 per cent of germination was obtained by layering fresh seeds out of doors over winter.

No satisfactory forcing agent has yet been found. A slight forcing effect of several acids, bases, and salts has been observed. The best of these forcing agents are nitrates and sulphates.

Although *Sambucus* seeds are probably injured by drying, that is not the only factor to be considered, since freshly gathered seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum.

As yet it has been impossible to approximate perfect germination, and much still remains to be learned concerning the conditions necessary to reach it.

RUBUS.—Dormancy is probably due to the high breaking strength of the endocarp. Seeds treated with concentrated sulphuric acid for 2 hours, then thoroughly washed, germinate readily on cotton, filter paper, or quartz sand.

The optimum temperature for germination lies between 20° and 25° C. Seeds germinate equally well in light or darkness. Naked seeds germinate poorly in soil. This may be due to the action of fungi, bacteria, or to other causes as yet unknown.

As a practical method for the germination of *Rubus* seeds, if one is not to resort to layering, the writer suggests the following: The seeds should be removed from the pulp as completely as possible. If the berries are crushed and then thrown into water most of the pulp can be floated off. The pulp still clinging to the seeds may be removed by allowing fermentation in water to take place or by treating the seeds with a 5 per cent solution of sodium hydroxide for 15–20 minutes, after which they should be thoroughly washed in running water. It is essential to dry the seeds for at least 24 hours, or the treatment with concentrated sulphuric acid which follows

will result in heating. The seeds should be left in the acid for approximately 2 hours.

In order to obtain uniform results it is advisable to use a large excess of acid and to prevent the seeds from gathering in clumps or layers. Frequent stirring is essential. By rubbing a few of the seeds in the palm of the hand from time to time it is possible to determine when the entire endocarp on a majority of the seeds has been carbonized. When this point is reached the acid should be drained away and the seeds thrown into an excess of cold water. It is advisable to change the water frequently or to put the seeds in running water, where they should be left for at least 15 minutes. When they are removed from the water they should be treated with an excess of a 5 per cent solution of sodium bicarbonate until bubbles cease to rise, after which they may be washed in running water for 15 minutes.

In order to remove the carbonized endocarp the seeds may be placed on filter paper and rubbed under the fingers. It is impossible to remove the endocarp if it has been allowed to become dry following the last washing.

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